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# **The Role of the Kidney in Glucose Homeostasis**

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Additional information is available at the end of the chapter

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## **1. Introduction**

It is only in recent years that the attention was drawn on the important role of the kidney in glucose homeostasis. Nevertheless, along with the liver, the kidney has an important role in ensuring the energy needs during fasting periods. This organ has a vital role in absorbing the entire quantity of the filtered glucose [1]. Having a glomerular filtration rate of 180 liters per day, it filters approximately 180 grams of glucose per day, bringing its contribution in maintaining normal fasting plasma glucose (FPG) levels [2]. The reabsorption of glucose is ensured by the sodium-glucose cotransporter (SGLT) 2, responsible for the reabsorption of 90% of glucose, and SGLT1, that reabsorbs the remaining glucose [3].

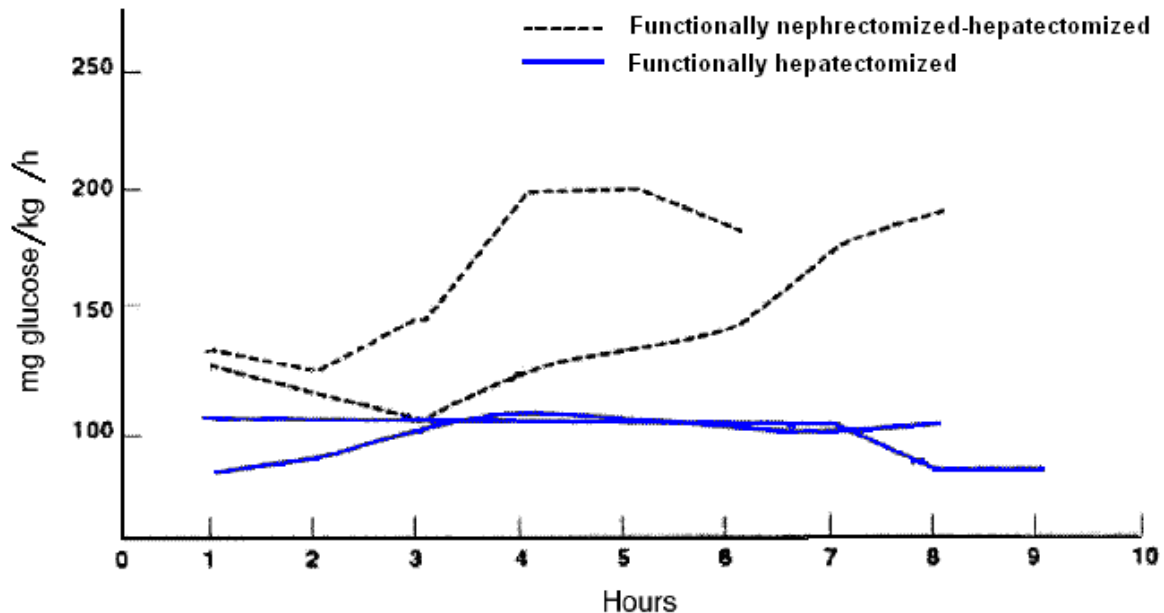
Despite the large amount of data regarding the implication of the kidneys in glucose homeostasis, this organ is often overlooked as a key player in glucose metabolism. But the awareness of the renal mechanisms of glucose control is likely to increase due to the development of new types of glucose-lowering drugs that target this metabolic pathway [4].

## **2. Short history**

### **2.1. Early non-human studies**

The first researchers in this field, Bergman and Drury brought the first clues about the involvement of the kidney in glucose homeostasis in 1938 [5]. They used the glucose clamp technique in order to maintain euglycemia in two groups of rabbits – one functionally hepatectomized and another one functionally hepatectomized and nephrectomized. In the group of hepatectomized and nephrectomized rabbits, the amount of glucose requested in order to maintain euglycemia was very high compared to the one required by the other group

[6] (Figure 1). These data led to the conclusion that the kidneys are an important source of plasma glucose [6].



**Figure 1.** Effect of nephrectomy on glucose needs for maintaining euglycemia in hepatectomized rabbits (Adapted from [6])

A few years later, the study was reproduced by Reinecke in rats. He also determined the arteriorenal venous glucose concentrations in the hepatectomized rats. He found that the glucose levels in renal vein exceeded the arterial levels when the animals became hypoglycemic proving that, under these conditions, the kidneys can release glucose into the circulation [7].

In 1950, Drury et al. injected  $^{14}\text{C}$ -labeled glucose into rats that had been hepatectomized or hepatectomized and nephrectomized. His experiment indicated that the kidney represents the source of the glucose produced endogenously and released into the circulation after hepatectomy [8].

In other experiments, Teng proved that the renal cortex of the animal models with diabetes released glucose at a very high rate, but treatment of these animals with insulin could reverse this effect. A few years later, in 1960, Landau was able to prove, having a similar model, that gluconeogenesis from pyruvate was increased by the diabetic kidney [6].

In several experiments, Krebs tried to characterize the substrates that the kidney uses for gluconeogenesis [9], the efficiency of the renal gluconeogenesis in several species [10], and some aspects of the regulation of renal gluconeogenesis [11]. He could also demonstrate that the kidney present a greater amount of gluconeogenic enzymes than the liver, and due to the comparable blood flows (therefore comparable provision of gluconeogenic precursors), Krebs argued that the kidney might be a gluconeogenic organ in vivo as important as the liver [11].

## 2.2. Early human studies

Studies about human renal glucose metabolism started in the late 1950s. They tried to measure the differences of glucose concentrations between arterial and renal venous blood. By not taking into consideration that the kidney is able to produce and consume glucose in the same time, the fact that many researchers found little or no differences between arterial and venous glucose values led to the conclusion that the kidneys are not able to release glucose [6].

In the mid 1960s, Aber et al. [12] found that kidney can release glucose in patients with pulmonary disease and the quantity of glucose is negatively correlated with arterial pH explaining why the greater the acidosis, the greater the renal glucose release. Several years after, Owen et al. [13] indicated that renal glucose release is increased in very obese patients who fasted for several weeks. These data led to the current textbook idea that the liver is the only source of glucose, in general, except after prolonged fasting or under acidosis.

On the other hand, in subjects that undergo liver transplantation, it may still be observed after removal of the liver, endogenous glucose production [14]. Shortly after the removal of the liver, the production of endogenous glucose decreased only by 50% (Joseph et al.) [14]. Recent research using isotopic measurements have indicated that the kidney can release significant quantities of glucose in postabsorptive normal volunteers.

## 3. The involvement of kidneys in glucose homeostasis

The plasma glucose concentration is determined by the amount of glucose synthesized, and the one removed from the circulation and metabolized. This concentration must be maintained within a relatively narrow range despite the wide daily fluctuations in glucose ingestion and glucose demands in various tissues [4]. Other substrates such as free fatty acids (FFAs), glycerol, lactate and ketone bodies have greater daily fluctuations. This can be explained by the need of the body to protect himself against hyper- and hypoglycaemia. Hyperglycaemia is associated with both chronic effects (such as nephropathy, retinopathy, neuropathy and premature atherosclerosis) and also acute complications (including diabetic ketoacidosis and hyperosmolar hyperglycaemic state that are associated with higher morbidity and mortality). Hypoglycaemia is also harmful because it can cause neurological events (including coma, seizures), cardiac arrhythmias and death [4].

The regulation of endogenous production of glucose is determined by hormonal and neural factors [15]. In the acute phase, glucoregulatory mechanisms involve insulin, glucagon and catecholamines and they can effect changes in plasma glucose levels in a matter of minutes. Insulin is able to suppress glucose release in both the kidney and liver by direct enzyme activation/deactivation and by reducing the availability of gluconeogenic substrates. Glucagon has no effect on the kidneys, but it stimulates glycogenolysis and gluconeogenesis in the liver [16]. Catecholamines also have multiple acute actions. They can stimulate renal glucose release and glucagon secretion and inhibit insulin secretion [4].

The kidneys are involved in maintaining glucose homeostasis through three different mechanisms: gluconeogenesis; glucose uptake from the blood for its own energy requests and reabsorption into the general circulation of glucose from glomerular filtrate in order to preserve energy [4].

### 3.1. Renal gluconeogenesis

From the point of view of glucose utilization, the kidney is considered as 2 separate organs; the renal medulla is characterized mainly by glucose utilization and the renal cortex is responsible for glucose release. The separation of these activities represents the consequence of differences in the distribution of numerous enzymes along the nephron. The cells in the renal medulla can use only glucose for their needs (like the brain) and they have enzymes capable of glucose-phosphorylation and glycolysis. They can therefore phosphorylate important amounts of glucose and accumulate glycogen but, because these cells do not have glucose-6-phosphatase or any other gluconeogenic enzymes, they are unable to release glucose into the bloodstream. Moreover, the cells in the renal cortex have gluconeogenic enzymes and they can produce and release glucose into the circulation. However these cells cannot synthesize glycogen because they have little phosphorylating capacity [6].

After a 16-h overnight fast, approximately  $10 \mu\text{mol}/(\text{kg}/\text{min})$  of glucose is released into the circulation [17]. Almost 50% of this is the result of glycogenolysis from the liver stocks and the other half is produced by liver and kidney gluconeogenesis. The renal cortex (like the liver) contains gluconeogenic enzymes and it can synthesize glucose-6-phosphate from precursors (lactate, glutamine, glycerol and alanine). Because it contains glucose-6-phosphatase, it is able to release glucose into the blood stream [18] and the human liver and kidneys are the only organs that can perform gluconeogenesis. Therefore, after an overnight fast, the liver produces 75–80% of glucose released into the circulation and the remaining 20–25% is derived from the kidneys [4].

Several studies have indicated that human kidneys and liver provide approximately the same amounts of glucose through gluconeogenesis in postabsorptive period. If the duration of fasting is increased, the glycogen stores are depleted and gluconeogenesis produces all the glucose released into circulation.

An important aspect is that kidney and liver use different gluconeogenic precursors and several hormones have different effects on their release of glucose. Lactate represents the predominant gluconeogenic precursor in both organs, but regarding the aminoacids, the kidney prefers to use glutamine, whereas the liver preferentially uses alanine [19]. Insulin can suppress glucose release in both organs with almost comparable efficacy [20], whereas glucagon stimulates hepatic glucose release only [21]. Catecholamines normally have a direct effect only on renal glucose release [22], but their effect on both hepatic and renal glucose release may be indirect by increasing the quantity of gluconeogenic substrates available and by suppressing insulin secretion. Other hormones, such as growth hormone, cortisol and thyroid hormones can stimulate hepatic glucose release over a great period of time [15]. Their effects on the kidneys regarding glucose release in humans are not completely deciphered.

In the postprandial state the situation changes significantly. Postprandial glucose levels in the plasma are determined by insulin and glucagon levels. After glucose ingestion, plasma glucose levels reach the peak in 60–90 minutes and they return to post-absorptive levels in almost 3–4 h. The plasma insulin increases four times and the plasma glucagon levels decrease by 50% [15]. Meyer et al. indicated that endogenous glucose release is reduced by almost 60% and hepatic glycogenolysis drops to zero in the 4- to 6-h period after meal ingestion [23]. This is happening because this period determines the refilling of hepatic glycogen stores and inhibition of endogenous glucose release is able to limit postprandial hyperglycaemia. There is also a reduction in hepatic gluconeogenesis by 82% and glucose molecules generated through hepatic gluconeogenesis are also directed into hepatic glycogen, not only released in the circulation.

Renal gluconeogenesis can increase by approximately twofold and it can represent ~60% of endogenous glucose production in the postprandial state [24]. This mechanism is believed to facilitate the repletion of glycogen stocks in the liver.

A new concept of hepatorenal glucose reciprocity emerged from the differences observed in regulation and interchange between renal and hepatic glucose release [24]. This concept refers to the facts that a pathological or physiological reduction in glucose release by kidney or liver determines a compensatory increase in glucose release of the other one (liver or kidney) in order to avoid hypoglycaemia. This situation occurs in the anhepatic phase during liver transplantation, prolonged fasting, meal ingestion, acidosis and insulin overdoses in diabetes mellitus [24].

### **3.2. Glycogenolysis**

Glycogenolysis is the breakdown of glycogen to glucose-6-phosphate and a hydrolysis reaction (using glucose-6-phosphatase) in order to free glucose. The liver is the only organ that contains glucose-6-phosphatase. So, the cleavage of hepatic glycogen releases glucose, while the cleavage of glycogen from other sources can release only lactate. Lactate, that is generated via glycolysis, is often absorbed by other organs and helps regenerating glucose [6].

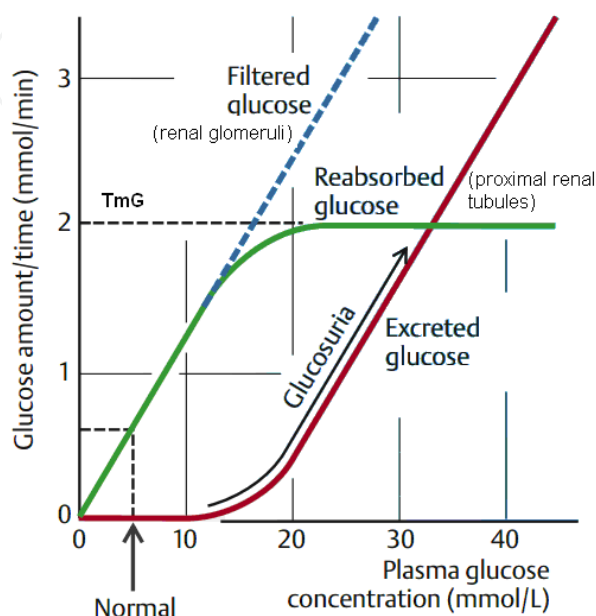
### **3.3. Glucose reabsorption**

Apart from the important role in gluconeogenesis and the role of renal cortex in glucose uptake, the kidneys contribute to glucose homeostasis by filtering and reabsorbing glucose. In normal conditions, the kidneys can reabsorb as much glucose as possible, the result being a virtually glucose free urine. Approximately 180 grams of glucose are filtered by the glomeruli from plasma, daily but all of this quantity is reabsorbed through glucose transporters that are present in cell membranes located in the proximal tubules [24].

These glucose transporters have a limited capacity of reabsorption. If this capacity is exceeded, glucose usually appears in the urine. The tubular maximum for glucose (TmG), the term used for the maximum capacity, can vary from 260 to 350 mg/min/1.73 m<sup>2</sup> in healthy subjects. It corresponds to blood glucose levels of 180-200 mg/dL [24]. When the blood glucose is very high and the TmG is reached, the transporters cannot reabsorb all the glucose and glucosuria



occurs (Figure 2). Nevertheless, there can be slight differences between the nephrons and the inaccurate nature of biological systems may potentially lead to the development of glucosuria when blood glucose is below  $T_mG$ . Glucosuria may occur at lower plasma glucose levels in certain conditions of hyperfiltration (eg. pregnancy), but as a consequence of hyperfiltration and not of significant hyperglycemia [25].



**Figure 2.** Renal glucose handling.  $T_mG$ , transport maximum for glucose. Adapted from [26]

In a given day, the kidneys can produce, via gluconeogenesis, 15–55g glucose and it can metabolize 25–35g glucose. Regarding the glucose metabolic pathways, it is obvious that renal reabsorption represents the main mechanism by which the kidney is involved in glucose homeostasis. Therefore, the change in tubular glucose reabsorption may have a considerable impact on glucose homeostasis [4].

### 3.3.1. Renal glucose transporters

Glucose is a polar compound with positive and negative charged areas; therefore it is soluble in water. Its transport into and across cells is dependent on two specialized carrier protein families: the GLUTs (facilitated glucose transporters) and the SGLTs (sodium-coupled glucose cotransporters). These transporters are responsible for glucose passage and reabsorption in several tissue types, including the proximal renal tubule, blood-brain barrier, small intestine [27]. GLUTs are responsible for the passive transport of glucose across cell membranes, in order to equilibrate its concentrations across a membrane. SGLTs, on the other hand, are involved in active transport of glucose against a concentration gradient by means of sodium-glucose cotransport [27].

There are six members of the SGLT family indicated in Table 1.

Co-transporter	Substrate	Tissue distribution
SGLT1	Glucose, galactose	Intestine, kidney, heart, trachea, brain, testis, prostate
SGLT2	Glucose	Kidney, brain, liver, muscle, heart
SGLT4	Glucose, mannose	Intestine, kidney, uterus, pancreas, liver, brain, lung, trachea
SGLT5	Unknown	Kidney
SGLT6	Glucose, myoinositol	Brain, kidney, intestine
SMIT1	Glucose, myoinositol	Brain, heart, lung, kidney

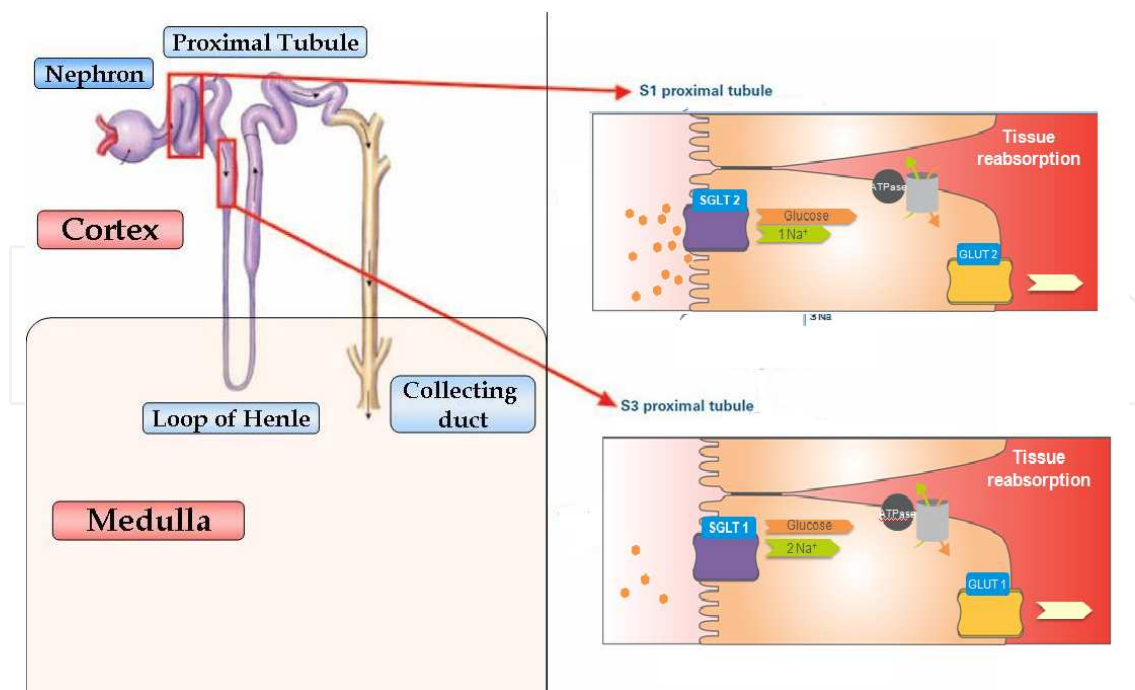
**Table 1.** The sodium glucose co-transporter family (adapted from [27])

SGLT2 is considered the most important because, based on animal studies, it is responsible for the reabsorption of 90% of the glucose filtered at the glomerulus [24]. The other 10% of glucose reabsorbed in the proximal tubule is ensured by SGLT1. Of the family of GLUT proteins expressed in the kidneys, GLUT2 is the major transporter and it releases into circulation the glucose reabsorbed by SGLTs in the proximal tubular cells [28].

The renal glucose transport was investigated by analyzing the gene mutations within SGLT family. These can lead to several inherited diseases presenting renal glucosuria that include familial renal glucosuria (FRG) and glucose-galactose malabsorption (GGM). FRG represents an autosomal recessive or autosomal dominant disorder caused by several SGLT2 mutations. Its main characteristic is persistent glucosuria without hyperglycemia or renal tubular dysfunction. Most of the patients with FRG do not have any clinical manifestations; this is why FRG is not commonly described as a “disease” but as a condition known as benign glucosuria. Nevertheless, there is a severe form of FRG, known as type O, where mutations of the SGLT2 gene lead to a complete lack of renal tubular glucose reabsorption. This condition is still associated with a good prognosis. Due to the fact that FRG is mainly asymptomatic, subjects with this condition are discovered through routine urinalysis [24].

GGM represents a more serious disease. It is inherited autosomal recessive and is caused by mutation of the SGLT1 transporter. Its main characteristics are represented by intestinal symptoms. They appear in the first few days of life and determine glucose and galactose malabsorption. The consequences are severe; diarrhea and subsequent dehydration may become fatal unless a special diet (glucose- and galactose-free) is initiated. Some patients with GGM may present glucosuria but it is typically mild, and some other subjects have no sign of urinary glucose excretion. This confirms that SGLT1 has a minor role in renal reabsorption of glucose [24]. The mutations involving the GLUT family are associated with more severe consequences, because these transporters are more widespread throughout the major organ systems. SGLT2 and SGLT1 are located mainly in the renal system, but GLUT2 is present almost everywhere in the organism, having an important role in glucose homeostasis through its involvement in intestinal glucose uptake, renal reabsorption of glucose, and hepatic uptake and release of glucose [24].





**Figure 3.** Glucose filtration and reabsorption in the proximal tubule of the kidney (adapted from [28])

Direct *in vivo* experiments of Vallon et al. on gene targeted mice lacking *Sglt2* gene, demonstrated that the SGLT2 protein is responsible for all glucose reabsorption in the proximal tubule and for the bulk of glucose reabsorption in the kidney overall [29]. According to this study, in wild-type mice,  $99.7 \pm 0.1\%$  of fractional glucose is reabsorbed and in *Sglt2*<sup>-/-</sup> mice (not expressing SGLT2), only  $36 \pm 8\%$  is reabsorbed. It was also found that in *Sglt2*<sup>-/-</sup> mice, even if SGLT1 glucose reabsorption is increased (SGLT1 transporters reach their transport maximum), up regulation of SGLT1 expression does not occur (both SGLT1 mRNA and protein expression are reduced by  $\sim 40\%$ ) when the amount of glucose in proximal tubule is increased. The results of the study of Gorboulev et al. [30] are in correspondence with those of Vallon et al., indicating that wild-type mice do not use the maximal transport capacity of SGLT1 at normoglycemic conditions but when glucose load to the SGLT1 is increased (for instance, diabetes and SGLT2 inhibition), SGLT1 may operate at full transport capacity [30].

Molecular structure of SGLTs has been studied thoroughly on SGLT1, which is the first described member of SGLTs family [31]. SGLT2 is 59% identical to SGLT1 and has almost the same architecture. Its secondary structure consists of 14-transmembrane helices (TM1–TM13) with both the NH<sub>2</sub> and COOH termini facing the extracellular side of the plasma membrane [32]. The first kinetic model of Na<sup>+</sup>/glucose co-transporters was proposed by Parent et al. [33].

#### 4. The kidney in diabetes mellitus

All the metabolic pathways regarding the involvement of the kidney in glucose homeostasis are modified in subjects with diabetes mellitus. Subjects with type 2 diabetes mellitus (T2DM)

have an increased renal release of glucose into the circulation in the fasting state [34]. Although one can think that the liver determines increased glucose release into the circulation in diabetes, the liver and the kidneys have comparable increase in renal glucose release (2.60 and 2.21  $\mu\text{mol}/(\text{kg min})$ ). The kidney can increase its glucose production with 300% compared with the liver that can increase gluconeogenesis only by 30%. Gluconeogenesis, in the kidney, could explain this glucose increase, in the fasting state [34].

In postprandial state, renal glucose release is greater increased in subjects with T2DM than in people without glucose metabolism abnormalities [35]. Meyer et al. studied systemic glucose appearance in subjects with T2DM and individuals with normal glucose tolerance over several hours following ingestion of 75 g glucose. They found that it was significantly greater in diabetic patients than in normal subjects ( $100.0 \pm 6.3$  vs.  $70.0 \pm 3.3$  g;  $p < 0.001$ ). The result was determined by a higher endogenous glucose release because the general appearance of ingested glucose was only 7 g greater in the subjects with DM. Almost 40% of the increased endogenous glucose release was caused by increased renal glucose release [35]. This fact was determined mainly by impaired suppression of endogenous glucose release and secondary by reduced initial splanchnic sequestration of ingested glucose. This effect is expected in diabetic patients that have decreased postprandial insulin release and insulin resistance, taking into account that renal glucose release is regulated by insulin [4].

Both renal glucose uptake and glucose production are increased in both the postprandial and post-absorptive states in diabetic patients [35].

It is well known that glucosuria in diabetic patients occurs at different plasma glucose levels compared with the levels where glucosuria can occur in non-diabetic individuals [36]. This is determined by the increased glucose reabsorption in subjects with diabetes mellitus. Therefore, the  $T_m$  for glucose is increased and glucosuria may occur at higher than normal blood glucose levels. Several studies indicated that the  $T_m$  increased from near 350 mg/min in subjects with normal glucose tolerance to approximately 420 mg/min in subjects with diabetes mellitus [36].

As an evolutionary process, the kidney was able to develop a system in order to reabsorb all of the filtered glucose in order to conserve energy especially at a time when energy intake was reduced. Therefore, this may be considered as an adaptive response as the SGLT2 transport increases in response to hyperglycaemia. But, in subjects with diabetes this adaptive response is considered maladaptive, and glycosuria occurs only at very high plasma glucose levels. Thus, instead of allowing the kidneys to excrete excess of glucose, SGLT2 transporters help maintain a higher plasma glucose concentrations [1].

Human and animal studies of renal cells have demonstrated enhanced expression of SGLT2 transporters [37]. Factors like hyperglycaemia, albumin and angiotensin II have been reported to increase the expression of SGLT2 in T2DM [37].

It has also been demonstrated that acidosis increases renal gluconeogenesis and impairs hepatic gluconeogenesis [38]. Therefore one can speculate that the kidney represents an important factor that accelerates gluconeogenesis in diabetic ketoacidosis. Moreover, the exaggerated increase in renal glucose release can be the result of the insufficient suppression of endogenous glucose release postprandial in diabetic patients [39]. These processes can

explain the quantity of glycogen stored in diabetic kidneys. A major part of the high renal glucose release found in subjects with diabetes may be determined by increased renal glycogenolysis [6].

## 5. Diabetic nephropathy

Diabetes represents the most common single cause of end-stage renal disease (ESRD) in the United States and Europe. There can be several factors responsible for this, including an increased prevalence of T2DM, longer life spans among patients with diabetes, and better recognition of kidney disease [40]. Comparing with subjects with type 1 diabetes mellitus, only a smaller fraction of those with T2DM develop ESRD, but due to the increased prevalence of T2DM, these individuals represent more than a half of those with diabetes on dialysis. There are numerous variables in progressing to nephropathy, including racial/ethnic variability because Native Americans, Hispanics and African Americans are at much greater risk of developing ESRD than non-Hispanic white subjects with T2DM [40].

The first clinical signs of nephropathy are represented by low, but abnormal, levels ( $\geq 30$  mg/day or  $20\mu\text{g}/\text{min}$ ) of albumin in the urine (previously referred to as microalbuminuria). The detection of albumin in the urine increases the risk of progression to persistent albuminuria, progressive decline in glomerular filtration rate (GFR), increased blood pressure and cardiovascular morbid-mortality. But because T2DM may be present for many years before diagnosis, a higher proportion of individuals with T2DM have microalbuminuria and overt nephropathy shortly after diagnosis. It is known that without treatment, 20-40% of patients with T2DM and microalbuminuria progress to overt nephropathy. Nevertheless, after 20 years from the onset of nephropathy, only 20% will have progressed to ESRD [40]. The explanation comes from the greater risk among subjects with diabetes and chronic kidney disease of dying from cardiovascular disease than progressing to ESRD.

Several clinical trials indicate that the onset and development of diabetic nephropathy may be significantly influenced by numerous interventions including tight glucose control and also use of angiotensin-converting enzyme inhibitors or angiotensin receptor blockers. This is the reason why annual screening for microalbuminuria is critical since it can lead to early diagnosis of nephropathy. Numerous studies, including the well-known Diabetes Control and Complications Trial and United Kingdom Prospective Diabetes Study indicated that intensive glycemic control represent a very important step in reducing the risk of developing microalbuminuria and overt nephropathy [40].

New data from the Action in Diabetes and Vascular Disease: Preterax and Diamicron MR Controlled Evaluation (ADVANCE) trial offers hope regarding the benefic effects of tight glucose control on decreasing the risk of nephropathy [41]. In the ADVANCE trial, after almost 5 years, subjects that were on intensive glycemic control had a 10% relative reduction in the combined outcome of major macrovascular and microvascular events. This was happening mainly because of a 21% relative reduction in the risk of developing nephropathy. The intensive glucose control is also important because it is associated with a 9% reduction in new

onset microalbuminuria [41]. Results of this study are of great importance since renal impairment is strongly associated with future risk of major vascular events, and death in patients with diabetes. Nevertheless, the role of modified renal glucose reabsorption in the progression of diabetic nephropathy is not elucidated [4].

## 6. Therapeutic implications

### 6.1. SGLT2 inhibitors

SGLT2 is highly specific for (several authors consider that it is found only in) the proximal tubules of the kidney, as compared to SGLT1 or GLUT2, therefore it is a preferred target for more specific renal pharmacologic interventions. Thus, the idea of interfering with the activity of the SGLT2 has gained much attention [2].

Inhibition of SGLT2 transporter 'resets' the reabsorption system by lowering the threshold for glycosuria, resulting the correction of the hyperglycemia [1]. Reduction of the blood glucose level can improve insulin resistance in muscle by increasing insulin signaling, GLUT4 and glycogen synthase activity [1].

The history of SGLT2 inhibitors starts in 1835 when phlorizin was found in the root bark of apple tree [42]. Many years after, it was found to be a non-specific SGLT1 and SGLT2 and it could increase glucosuria and reduce blood glucose levels and normalize insulin sensitivity in a pancreatectomized animal model of T2DM [43]. Nevertheless, it could not become a treatment for diabetes due to numerous side effects. Being non-selective and inhibiting SGLT1 at the intestinal brush border, it can cause serious problems regarding the absorption of dietary glucose. Inhibition of SGLT1 can result in glucose–galactose malabsorption and cause diarrhea, events that occur naturally in SGLT1 deficiency [44]. Moreover, in the intestine, phlorizin is poorly absorbed and is rapidly hydrolyzed to phloretin, a substance that blocks GLUT1, leading to disturbance in glucose uptake in several tissues [45]. Highly-specific inhibitors of SGLT2 have subsequently been developed in order to overcome some of these shortcomings.

Ellsworth et al [46] discovered a group of C-aryl glycosides that includes dapagliflozin [47] and canagliflozin [48]. They are resistant to degradation produced by  $\beta$ -glucosidase enzymes in the gastrointestinal tract. Moreover, dapagliflozin has a very high sensitivity for SGLT2 compared to SGLT1, blocking renal glucose reabsorption by almost 40–50%. Using this treatment, they can be excreted up to 80–85 g of glucose per day [47]. Clinical trials evaluating the treatment with dapagliflozin, either as monotherapy or in association with metformin or with insulin in subjects with T2DM have demonstrated its efficacy in reducing glucose and HbA1c levels [3]. Pharmacokinetics and bioavailability of dapagliflozin are not influenced by a high-fat meal and there are no reports regarding any interactions with several other drugs used in the treatment of T2DM [3].

Human trials analyzing canagliflozin are more limited than for dapagliflozin. It has been indicated that both drugs have similar therapeutic characteristics [3]. Canagliflozin could



induce an important, dose-dependent decrease in the mean renal glucose threshold to approximately 60 mg/dl (3.33 mmol/l) [49].

There are numerous other SGLT2 inhibitors including sergliflozin, remogliflozin, ipragliflozin and empagliflozin. Some of them, such as ipragliflozin and empagliflozin, are being tested in phase III trials and are promising very good results while other compounds have disappointed in clinical trials due to possible side effects (sergliflozin) or to susceptibility to hydrolysis by  $\beta$ -glucosidase enzymes (sergliflozin and remogliflozin) [3].

As already mentioned, patients diagnosed with FRG often gave higher urinary glucose excretion of almost 120 g per day. It remains unclear why treatment with SGLT2 inhibitors cannot achieve the same levels of glycosuria even when the maximal doses are used. Moreover, SGLT2-null mice can only reabsorb up to a third of the filtered glucose [29], but subjects taking dapagliflozin reabsorb ~50% at the highest doses. Moreover, the nonselective inhibitor phlorizin completely blocks reabsorption. One possible explanation may be that SGLT1 has a greater role in the kidney than it was previously imagined [50]. There are some theories that include antisense nucleotide technology to knock out SGLT2 in order to achieve a higher degree of blockade of glucose reabsorption than SGLT2 inhibition. Preliminary data in human subjects with T2DM with moderate or severe renal impairment indicate that SGLT2 inhibition determines proportionally less glycosuria than in subjects with preserved renal function [51]. These findings confirm that a low GFR in subjects with T2DM is accompanied by a comparable loss of tubular absorptive capacity that represents the anticipated consequence of nephron loss [3].

The approach of lowering hyperglycaemia in T2DM by blocking glucose reabsorption has many attractions. One of them is represented by the activity of SGLT2 inhibitors that is not dependent on pancreatic  $\beta$ -cell function, which deteriorates over time. This is the only class of drugs that present this mechanism of action. Other drugs such as the insulin secretagogues [glinides, sulphonylureas, dipeptidyl peptidase-4 (DPP-4) inhibitors and glucagon-like peptide 1 (GLP-1) agonists] and insulin sensitizers (thiazolidinediones and metformin) depend on insulin secretion. The insulin independence of their action indicates that the risk of hypoglycaemia is very low [6].

As a consequence, the liver can react to the induced glycosuria by increasing glucose release. The mechanism for increased liver glucose excretion is not well understood. The relative small decrease in plasma glucose but also insulin concentrations after massive glycosuria may stimulate endogenous glucose release. Other additional mechanisms are not excluded. Moreover, glucose output is usually not decreased enough to attain and maintain normal glucose values in patients with T2DM treated with SGLT2 inhibitors [52]. Adaptation of glucose metabolism to massive glycosuria needs further investigation.

Osmotic diuresis accompanies glycosuria. It is usually detected an increase in urine output with acute SGLT2 inhibition; while chronic administration of SGLT2 inhibitors is accompanied by an excess urine volume of 200–600 ml per day. As a consequence, haematocrit increases are noted but they are moderate and clinical signs of volume depletion, such as tachycardia and orthostatic hypotension, are rarely met [52].

SGLT2 inhibitors determine glucose and sodium reabsorption blocking and natriuresis also occurs. Changes in serum sodium concentration are not frequent with chronic SGLT2 inhibition because at the nephron level, reduced sodium reabsorption in the proximal segment determines the increase of sodium delivery to the juxtaglomerular apparatus, and the inhibition of the renin-angiotensin-aldosterone system (RAAS) occurs. In experimental diabetic rats fed a high-salt diet [53], SGLT2 inhibition could prevent blood pressure increase. This effect may be countered by an activation of the RAAS if volume depletion appears as a consequence of excessive diuresis. SGLT2 inhibition in patients with T2DM also determines the reduction of blood pressure levels (by 2–5 mmHg) [52]. The possible explanations may be the enhanced natriuresis and RAAS deactivation [3]. Because most of the individuals with T2DM also present high blood pressure, this effect is of great importance in clinical practice.

Several phase III clinical trials of dapagliflozin reported the decrease of serum uric acid concentrations [54]. Sodium and urate are handled together in several physiological circumstances, and also in response to several drugs such as diuretics and antihypertensives. Several sodium-dependent phosphate transporters may also excrete urate into the urine. Therefore, the excretion of urate determined by SGLT2 inhibitors is explained by this mechanism. GLUT9 might represent an alternate explanation. GLUT9 represents an antiporter that exchanges glucose for uric acid; his two isoforms act together to reabsorb glucose from the tubule lumen in exchange for uric acid [55].

Another important effect of SGLT2 inhibition is weight loss. Clinical trials in patients with T2DM have reported a decrease of 2.5–4.0% of body weight [52]. At first, this weight loss is predominantly due to fluid depletion, but soon after that appears the loss of subcutaneous and visceral depots of adipose tissue. This effect is caused by an important caloric loss through the urine. Nevertheless, body weight loss remains constant after several months of treatment [3].

Clinically, the most frequent and undesired effect of SGLT2 inhibitors is represented by high incidence of genitourinary infections. These infections were observed more frequent in women than in men taking SGLT2 inhibitors and tend to occur in susceptible subjects; these include postmenopausal women, history of urinary tract infections or poor hygiene. Interestingly, studies with dapagliflozin in addition to metformin reported a not significant difference in incidence of genitourinary infections between individuals in the placebo and treatment groups [56], while in subjects receiving dapagliflozin in addition to insulin, the difference was significant [57]. This might explain a possibly increased risk of this adverse effect in patients with advanced T2DM (when immune function may be defective) [3].

The incidence of genitourinary infections tends to decrease in time, with long-term treatment, when the adaptation to the treatment is installed or exclusion of susceptible individuals over time appears. More important, infections of the upper urinary tract, that tend to be more severe than those of the lower urinary tract, are not frequent, although the reported patient exposure is presently too limited to rule out this adverse event [56].

Another reported event was a very small, but consistent, increase in PTH levels (<2.0 ng/l) together with increased plasma phosphate concentration. The increased PTH might indicate a mild form of secondary hyperparathyroidism but the available studies so far offer very few



data regarding the long-term effects of SGLT2 inhibitors on bone metabolism, making room for other clinical studies on this important issue.

There have been reports regarding several cases of bladder cancer and breast cancer, in subjects with T2DM receiving treatment with dapagliflozin [58]. Trials with large numbers of patients with different SGLT2 inhibitors are required to assess any associated increased risks of breast or bladder cancer [3].

Theoretical safety and tolerability concerns also include impairment in renal function [54]. Although, until now, there are no data indicating that the SGLT2 inhibitors would determine or be responsible for deterioration of renal function, the few clinical studies investigating these drugs have relatively short duration (6-12 months). Moreover, several authors are speculating that SGLT2 inhibitors may play an important role in preventing diabetic nephropathy. First, improved glycaemic control decreases the risk of diabetic nephropathy and other diabetic complications [40]. Second, by increasing the quantity of sodium in the juxtaglomerular apparatus, the use of SGLT2 inhibitors may determine a protective effect on the kidney, independently of glucose decreased.

In T2DM, the high quantity of glucose and sodium absorbed in the proximal tubule reduces the quantity of sodium to be delivered to the juxtaglomerular apparatus. Thus, the glomerulo-tubular feedback reflex is activated; this leads to high renal plasma flow, increased intra-glomerular pressure and elevated GFR. All these processes can induce normal salt delivery to the juxtaglomerular apparatus; however this can result in increased intra-glomerular pressure. All these alterations in renal hemodynamic lead to renal hypertrophy and eventually the result is represented by diabetic nephropathy [59]. SGLT2 inhibitors may prevent diabetic nephropathy by inhibiting the glomerulo-tubular feedback reflex and, therefore increasing sodium delivery to the distal nephron [1]. Nevertheless, this therapy is contraindicated in patients with estimated GFR (eGFR)  $<45$  mL/min/1.73 m<sup>2</sup> and must be used at lower doses at eGFRs of 45-60 mL/min/1.73 m<sup>2</sup> [60]. New clinical trials are expected to evaluate the efficacy and safety of SGLT2 inhibitors.

The pathogenesis of type 2 diabetes combines numerous defects in many tissues. Therefore, there is no single antidiabetic drug that can compensate all the metabolic disturbances, and a good treatment for diabetes will require the use of multiple drugs in combination. Having a unique pharmacokinetic and a special mechanism of action, the SGLT2 inhibitors can be used not only as monotherapy [61] but also in combination with currently available antidiabetic agents [62,63].

## 7. Conclusions

Although not traditionally discussed, the kidneys play a very important role in maintaining glucose homeostasis by gluconeogenesis and glucose reabsorption, the latter being mediated by active (SGLT) and passive (GLUT) transporters. Only recently, excessive renal glucose reabsorption was taken into consideration regarding its importance in the physiopathology of

T2DM. In hyperglycemia, the kidneys may play an exacerbating role by reabsorbing excess glucose, bringing their contribution to chronic hyperglycemia. Knowing the kidneys' role in glucose homeostasis and the effect of glucose dysregulation on the kidneys is very important for the optimal management of T2DM and prevention of associated renal complications. The numerous metabolic defects found in T2DM imply the use of several therapies. SGLT2 inhibitors represent a new promising class of drugs for the treatment of T2DM.

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